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Low Temperature Treatments Induce an Increase in the Relative Content of Both Linolenic and Δ^3-*trans*-Hexadecenoic Acids in Thylakoid Membrane Phosphatidylglycerol of Squash Cotyledons

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The effect of low temperatures on the fatty acid composition of phosphatidylglycerol (PG) in thylakoid membranes, in particular on the ratios of nmol% 16:1(3*t*) (mg fresh weight)$^{-1}$ of cotyledons and nmol 16:1(3*t*) (mg chlorophyll)$^{-1}$ were measured during squash seedling growth. Plants were germinated and grown for one day at 30°C, then were either kept at 30°C (control plants) or transferred to low temperatures (18, 14 or 10°C). When plants were transferred from 30°C to low temperatures, the increase in fresh weight was gradually limited. The lower the temperature, the smaller was the fresh weight. In contrast, the relative content of 16:1(3*t*) and 18:3, as well as the ratios of nmol 16:1(3*t*) (mg chlorophyll)$^{-1}$ and mol% 16:1(3*t*) (mg cotyledon fresh weight)$^{-1}$ increased indicating that the increase of fresh weight and chlorophyll was more sensitive to low temperature than PG desaturation in thylakoid membranes. Furthermore, low temperatures induced an increase in 16:1(3*t*) and 18:3 (the final products of PG synthesis) at the expense of 16:0 and 18:1 (the initial products of PG synthesis). However, within a range of temperature from 10 to 18°C, the extent of these changes (nmol% of 18:3 or 16:1(3*t*) per day) was gradually limited by lower temperatures. We therefore propose that low temperatures inhibit both fatty acid synthesis and desaturation activities. However, at low temperatures the fatty acid synthesis is likely to be more strongly inhibited than the desaturation activities, thus explaining the observed increase in the relative content of PG-18:3 and PG-16:1(3*t*). Results are discussed in terms of the mechanism which could be involved in the metabolism of PG in squash cotyledons.

Key words: Chilling temperature — *Cucurbita moschata* Durch — Development — Linolenic acid — Phosphatidylglycerol — Δ^3-*trans*-hexadecenoic acid.

Low temperatures induce many changes in plant green tissues of chilling-sensitive plants. Among these, growth restriction (Lyons 1973, Brüggemann et al. 1992, Ulrich and Bournérias 1992), leaf chlorosis (Hugly et al. 1990, McWilliam and Naylor 1967), and an increase in membrane lipid unsaturation (Kodama et al. 1995) have been observed in a variety of plants. It is considered that the former two parameters are reliable symptoms of chilling injury (Lyons 1973, Hugly et al. 1990), while lipid unsaturation is one of the factor maintaining the membrane fluidity at low temperature for the survival of plants under chilling conditions (Kodama et al. 1995, Vigh et al. 1993).

At a critical temperature, plant growth stops. This is the so-called zero of vegetation, e.g. from 6 to 10°C for maize, from 10 to 13°C for rice and near 0°C for the winter cereals (Ulrich and Bournérias 1992). Chlorosis is a common symptom of chilling injury in chilling-sensitive species such as cucumber (Hasselt 1972), sorghum (Slack et al. 1974) and maize (McWilliam and Naylor 1967, Schapendonk et al. 1989). It was reported that chill-induced chlorosis in maize seedlings is partly the result of two metabolic blocks in the porphyrin pathway leading to chlorophyll synthesis (Hodgins and Huystee 1986). The chill-induced growth restriction and chlorosis are usually used to determine the chilling sensitivity of plants (Schapendonk et al. 1989, Hugly et al. 1990).

Changes in the lipid composition in response to low temperature have been observed to occur in a variety of chilling-sensitive and -resistant plants. In general, there is an increase in 18:3 when plants are grown at low temperature. If the desaturation of lipid induced by low temperature is the factor which maintains the membrane fluidity at low temperature, the desaturation of phosphatidylglycerol should be very important. First, of all thylakoid membrane lipids, only PG contains high levels of disaturated molecular species (Murata 1983). Second, the relative content of disaturated molecular species of PG is significantly higher in chilling-sensitive plants than in chilling-resistant ones, with only a few exceptions (Murata 1983, Roughan 1985). Several attempts have been made to determine whether the unsaturation of fatty acids contributes to the tolerance ability of plants at low temperature (Somerville 1995, Murata and Wada 1995). However, the results are controversial. Murata et al. (1992) demonstrated by genetic manipulations that changes in fatty acid unsaturation of PG can alter plant chilling sensitivity. Furthermore, in a

Abbreviations: DGDG, digalactosyldiacylglycerol; m:n, fatty acid containing m carbons and n cis double bonds; MGDG, monogalactosyldiacylglycerol; PG, phosphatidylglycerol; SQDG, sulfoquinovosyldiacylglycerol; 14:0, myristic acid; 16:0, palmitic acid; 16:1(3*t*), Δ^3-*trans*-hexadecenoic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid.

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mutant of *Arabidopsis* (*fab1*), leaf PG contains 43% of high-melting point molecular species, a percentage higher than in many chilling-sensitive plants. However, the mutant is completely unaffected, when compared with wild-type controls, by a range of low-temperature treatments that rapidly led to the death of other chilling-sensitive plants (Wu and Browse 1995).

The mechanisms leading to temperature-induced changes in the lipid composition of thylakoid membranes are not yet fully elucidated. We have previously shown that thylakoid membranes of squash cotyledons grown at 20°C (compared with those from plants grown at 30°C), a non-injurious temperature, not only contain higher level of 18:3, but also 16:1(3*t*) in PG. However, when squash plants having mature cotyledons were transferred from 30°C to 20°C, no significant changes are detected during 6 days (Xu and Siegenthaler 1996b). We therefore proposed that an increase in the level of the final desaturation products of PG at low temperature is likely to be the result of changes in the relative activities of the fatty acid synthesis and desaturase. To test this hypothesis, we studied the effect of low temperatures on the fatty acid composition of PG in thylakoid membranes, in particular on the relative content of 16:1(3*t*) based on the fresh weight of cotyledons and chlorophyll during growth of squash seedlings.

Our results indicate that the increase of cotyledon fresh weight and chlorophyll content are more sensitive to chilling temperature than the activity involved in PG desaturation during squash seedling growth. The observed increase of the relative level of PG-18-3 and -16:1(3*t*) induced by low temperatures appears to be the result of a relatively higher inhibition of fatty acid synthesis than of the desaturation reactions.

Materials and Methods

Plant materials and growth conditions—Squash plants (*Cucurbita moschata* Durch. cv. Shirakikuza) were grown from seeds in soil (Mio Plant Natura, Migros, Switzerland). The seeds were germinated in darkness at 30°C and the seedlings grown in controlled environment growth chambers equipped with both fluorescent and incandescent lights (Sanyo, Gallenkamp, U.K., Cabinet Model PG 660) under a 12 h photoperiod (photon flux density (PFD) : 100 μmol m^{-2} s^{-1}) at 30°C for one day. At the end of the first light period, control plants remained under the same growth conditions, whereas other seedlings were transferred to various temperatures (18°C/60% RH, 14°C/65% RH and 10°C/70% RH) with the same light regime. Chloroplasts were immediately isolated from cotyledons collected after the desired daily light period.

Isolation of thylakoid membranes—Cotyledons (about 20 g corresponding to 40 to 120 cotyledons depending on the period of harvest) were ground and chloroplasts, then thylakoids isolated according to the method described by Xu and Siegenthaler (1996b). The chlorophyll concentration was determined according to Bruinsma (1961).

Lipid extraction and purification—Lipids were extracted from thylakoid membranes according to Siegenthaler et al. (1989). Lipid classes were separated by thin layer chromatography (TLC) on silica gel plates (pre-coated silica gel plates, Merck 5626) in two dimensions. The first developing solvent was acetone/toluene/H_2O (91 : 30 : 8, by volume) and the second was chloroform/methanol/25% NH_3/H_2O (65 : 35 : 3 : 2, by volume). The plates were dried shortly in air and lightly sprayed with 0.01% primuline and viewed under UV light.

Determination of fatty acids—The individual thylakoid membrane lipids separated by TLC were transesterified with 5% H_2SO_4 in MeOH for 1 h at 85°C. The fatty acid methyl esters were separated on a Hewlett-Packard 5890 gas chromatography supplied with an hydrogen flame ionization detector and a capillary column FFAP (30 m; i.d. 0.53 mm). The column was isothermally run at 190°C and the detector was held at 230°C. Arachidic acid (from Sigma) was used as an internal standard.

Results

Effect of growth temperature on cotyledon fresh weight—To examine the effect of low temperatures on plant growth, the fresh weight of developing cotyledons is a good and simple criterion as illustrated in Fig. 1. Cotyledons reached their maximum fresh weight (about 430 mg per cotyledon) when they were grown at 30°C for only three days after the transfer (control plants). The cotyledon growth was greatly restricted by lower temperatures. When plants were transferred from 30°C to 18°C (a non-chilling temperature) the fresh weight of their cotyledons reached after 6 days of transfer the same level as those of control plants. When plants were transferred to 14°C (a temperature which is close to that causing chilling injury), the increase of cotyledon fresh weight was after 6 days about half of that of control plants. Meanwhile, no chilling sym-

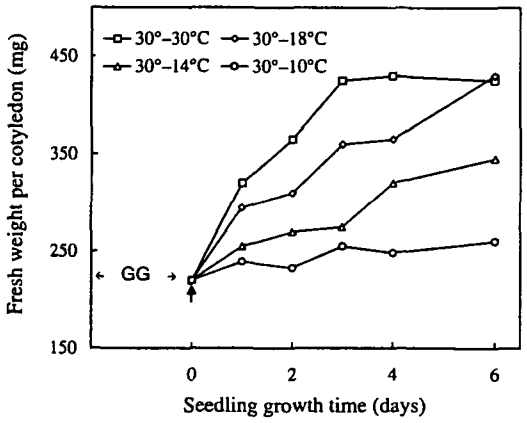

Fig. 1 The effect of low temperatures on the fresh weight of developing squash cotyledons. GG corresponds to 5 d of seed germination in darkness at 30°C, followed by one day of seedling growth at the same temperature with a 12 h photoperiod and a PFD of 100 μmol m^{-2} s^{-1}. At the end of this one day light period, seedlings were transferred from 30°C to 18, 14, or 10°C with the same light regime. The vertical arrow indicates the time at which seedlings were transferred to lower temperatures.

ptoms were observed. In contrast, when plants were transferred to 10°C (a chilling temperature), the cotyledon growth was almost stopped and the chilling symptoms (e.g. damage to the root with appearance of necrotic lesions) appeared after 6 days. The death of the plants began at the 6th day and all plants died after 8 days. Plants were also transferred from 30°C to 5°C but they survived only for 3 days.

Effect of growth temperature shift on the relative molar content of glycerolipids and fatty acid composition of glycolipids in thylakoid membranes—Preliminary results (not shown) revealed that the relative molar content of each of the four glycerolipid classes did not depend on the growth temperature and light energy as well as on the developmental stage of squash cotyledons. The relative levels of MGDG, DGDG, SQDG and PG were 53, 30, 6 and 11 mol%, respectively. In contrast, the fatty acid composition of glycerolipids underwent changes which were particularly marked in PG. The fatty acid composition of glycolipids (MGDG, DGDG and SQDG) were determined as a function of growth time (i.e. at 0, 1, 2, 3, 4 and 6 days) under four temperature shift conditions. For the sake of simplicity, only the results obtained at 0, 1, 2 and 4 days are presented (Table 1). Under all conditions, the content of 18:3 increased mainly at the expense of 18:2 and to a lesser extent of 18:1 and 18:0 in the three lipid classes. These changes occurred especially during the first two days of growth after the shift temperature. In contrast, no significant change was observed at the level of 16:0 during growth in the three glycolipids when the temperature remained constant (30°/30°C). However, when it was lowered, 16:0 content diminished progressively as a function of growth time in galactolipids but not in SQDG. The data confirm that growth temperature shift did not affect markedly the fatty acid composition of glycolipids and that the fatty acid composition of PG will be the interesting one to be considered.

Effect of growth temperature on the relative composition of fatty acids in thylakoid membrane PG—Low temperature treatments induce usually an increase in 18:3 of membrane lipids (Graham and Patterson 1982). We have previously found that in squash, this change occurs only in the developing cotyledons and furthermore that low temperature (20°C compared to 30°C) induces an increase in the content of 16:1(3*t*) in PG during growth (Xu and Siegenthaler 1996b). Figure 2 shows the effect of different low temperatures on the 18:3 content of PG in developing cotyledons of squash. When plants were isothermally grown at 30°C, PG contained about 8 mol% of 18:3, a level which remained constant during growth. When plants were transferred from 30°C to lower temperatures, an increase in 18:3 was detected after two d. After six d, the 18:3 content reached about 12 mol%, which corresponded to an increase of about 50% compared to that of control plants. Fig. 2 also shows that between the 2nd and 4th day, the increase of 18:3 levels was gradually limited by low temperatures. The effect of low temperatures on the 16:1(3*t*) content in thylakoid PG of squash cotyledons is illustrated in Fig. 3. The 16:1(3*t*) level increased during cotyledon growth under all conditions. When plants were grown isothermally at 30°C, the relative content of 16:1(3*t*) increased from about 5 to 15 mol% during the first 3 d, then remained constant. Lower temperatures enhanced the 16:1(3*t*) content but the lower the temperature, the lesser was the enhancement.

The relationship between the relative content of 18:3 and the sum of the other C_{18}-fatty acids in thylakoid PG from cotyledon squash plants grown at various temperatures is shown in Fig. 4. For all transfer temperatures tested, the correlation between the content of 18:3 and the

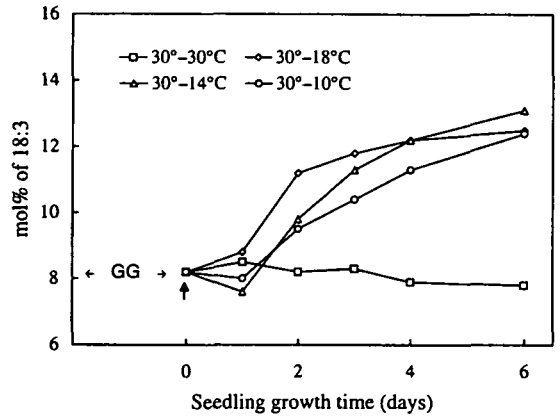

Fig. 2 The effect of low temperatures on the relative linolenic acid (18:3) content in thylakoid phosphatidylglycerol from developing squash cotyledons. Growth conditions and symbols are described in the legend of Fig. 1.

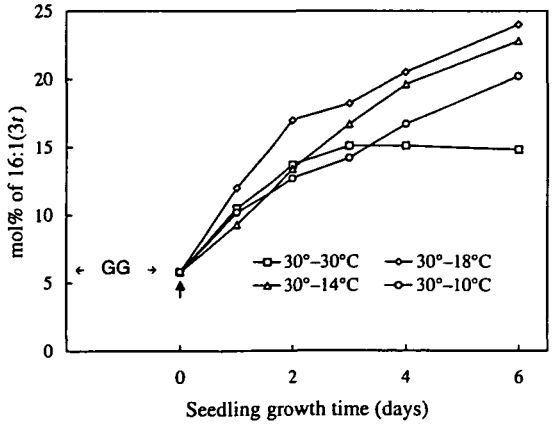

Fig. 3 The effect of low temperatures on the relative Δ^3-*trans*-hexadecenoic acid [16:1(3*t*)] content in thylakoid phosphatidylglycerol from developing squash cotyledons. Growth conditions and symbols are described in the legend of Fig. 1.

Table 1 Effect of temperature shift of squash plants on the fatty acid composition of thylakoid membrane glycerolipids (MGDG, DGDG, and SQDG)

Lipides	Temperature shift	Growth days	Fatty acid composition (mol%)				
			16:0	18:0	18:1	18:2	18:3
MGDG	30°-30°C	0	1.5	0.0	1.1	6.4	90.9
		1	1.6	0.0	0.5	2.9	95.0
		2	1.7	0.0	0.6	2.8	94.9
		4	2.0	0.0	0.5	3.0	94.4
	30°-18°C	1	1.4	0.0	0.8	3.9	93.8
		2	1.2	0.0	0.3	2.1	96.4
		4	1.0	0.0	0.2	2.2	96.6
	30°-14°C	1	1.7	0.0	1.1	5.4	91.9
		2	0.9	0.0	0.2	1.9	97.0
		4	0.8	0.0	0.1	1.5	97.5
	30°-10°C	1	1.7	0.0	1.1	6.0	91.2
		2	1.4	0.0	0.6	2.7	95.4
		4	1.0	0.0	0.2	1.4	97.4
DGDG	30°-30°C	0	9.9	1.6	0.9	5.9	81.7
		1	9.4	0.9	0.7	3.6	85.5
		2	9.3	0.7	0.7	3.5	85.9
		4	9.4	0.5	0.2	3.2	86.7
	30°-18°C	1	9.6	1.2	0.9	5.2	83.0
		2	8.1	0.9	0.6	3.3	87.1
		4	6.7	0.3	0.4	1.5	91.1
	30°-14°C	1	9.3	1.5	1.0	4.3	83.9
		2	8.0	0.8	0.7	4.0	86.5
		4	7.0	0.5	0.4	2.4	89.7
	30°-10°C	1	8.4	1.2	0.9	4.2	85.2
		2	7.8	0.7	0.7	3.4	87.4
		4	7.2	0.4	0.4	2.2	89.8
SQDG	30°-30°C	0	26.6	6.4	3.2	1.3	50.6
		1	26.9	5.7	2.9	8.5	56.0
		2	27.6	4.7	2.9	7.5	57.3
		4	29.4	3.9	1.9	6.7	58.0
	30°-18°C	1	27.1	8.8	3.4	10.3	50.3
		2	25.3	6.0	2.8	6.9	59.0
		4	26.0	4.8	1.4	5.6	62.2
	30°-14°C	1	27.0	7.0	2.9	12.1	50.9
		2	25.7	8.1	3.1	8.1	55.1
		4	25.0	4.8	1.1	5.2	63.9
	30°-10°C	1	25.6	6.9	3.4	12.0	52.0
		2	25.5	5.5	2.5	9.3	57.2
		4	25.9	5.7	2.0	5.7	60.7

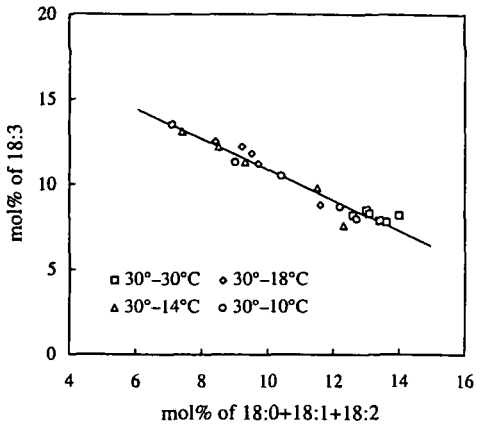

Fig. 4 Relationship between the relative content of 18:3 and the sum of 18:2, 18:1 and 18:0 in thylakoid phosphatidylglycerol from developing squash cotyledons. Plant growth conditions are described in the legend of Fig. 1. The equation of the straight line was $y=-1.02x+20.93$ ($r=0.98$).

sum of 18:2, 18:1 and 18:0 was linear and decreasing ($y=-1.02x+20.93$). Under all temperature conditions, an increase in 18:3 content occurred mainly at the expense of 18:1 and to a lesser degree of 18:2 and 18:0 (results not shown). A similar relationship was calculated for the C_{16}-fatty acid series (Fig. 5). Again, for all growth temperatures studied, the correlation between the content of 16:1(3*t*) and 16:0 was linear and decreasing ($y=-1.21x+87.05$). The excellent correlations between 18:3 and the sum of 18:2, 18:1 and 18:0 relative content ($r=0.98$) as well as between 16:1(3*t*) and 16:0 relative content ($r=0.99$) indicate that low temperatures did not affect the sum of the fatty acids within each of the C_{18}- and C_{16}-series.

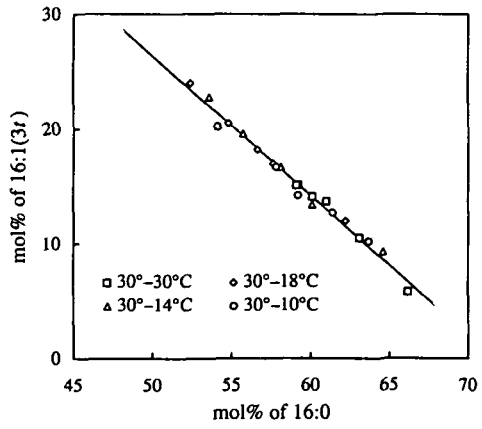

Fig. 5 Relationship between the relative content of 16:0 and 16:1(3*t*) in thylakoid phosphatidylglycerol from developing squash cotyledons. Plant growth conditions are described in the legend of Fig. 1. The equation of the straight line was $y=-1.21x+87.05$ ($r=0.99$).

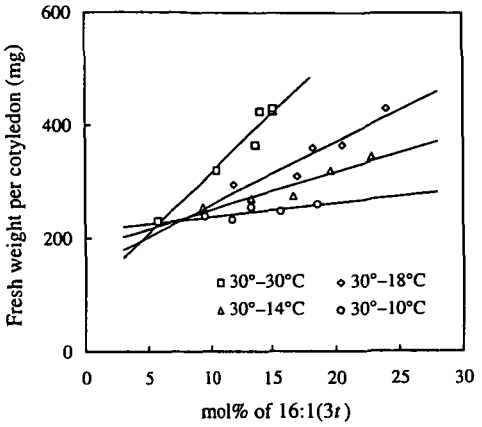

Fig. 6 Effect of low temperatures on the relationship between the fresh weight of developing cotyledons and the relative molar content of 16:1(3*t*) in phosphatidylglycerol of thylakoid membranes. Plant growth conditions are described in the legend of Fig. 1. The equation of the straight lines was $y=22.34x+87.50$ at 30°C; $y=11.26x+145.54$ at 18°C; $y=6.80x+181.77$ at 14°C; and $y=1.97x+203$ at 10°C.

Relationship between growth temperature and the slope expressing the fresh weight versus the content of 16:1(3t) in PG—During the growth of squash seedlings, the curves expressing the fresh weight of cotyledons (Fig. 1) and the relative content of 16:1(3*t*) [Fig. 3] displayed a similar pattern at each temperature. Therefore, a linear correlation between these two parameters should exist. The results of Fig. 6 show that each squash plant grown at a given temperature was characterized by its own ratio between fresh weight of cotyledons and the relative content of 16:1(3*t*) in thylakoid membrane PG. The equations of

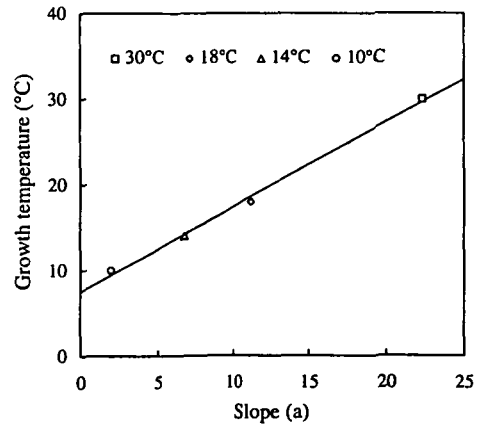

Fig. 7 Relationship between the growth temperature after the transfer of seedlings (30° → 18°, 14° or 10°C) and the slope (a) of the straight lines obtained in Fig. 6, i.e. $a=22.34$ for a transfer from 30°C → 30°C, $a=11.26$ (30° → 18°C), $a=6.80$ (30° → 14°C) and $a=1.97$ (30° → 10°C). The equation of the straight line is $y=0.99x-7.51$ ($r=0.99$).

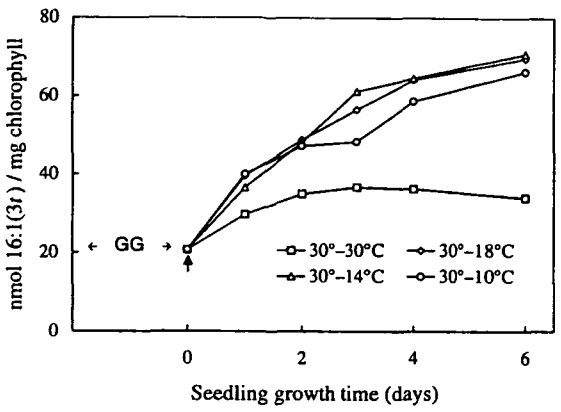

Fig. 8 Effect of low temperatures on the ratio of 16:1(3*t*) content in thylakoid phosphatidylglycerol and chlorophyll as a function of growth time in developping squash cotyledons. Growth conditions are described in the legend of Fig. 1. The vertical arrow indicates the time at which seedlings were transfered at lower temperatures.

the straight lines were y=22.3x+87.5 (at 30°C), y=11.3x+145.5 (at 18°C), y=6.8x+181.8 (at 14°C), y=2.0x+203 (at 10°C). The relationship between the growth temperature after the transfer of seedlings and the slope of the straight lines (see Fig. 6) is illustrated in Fig. 7. It can be seen that the lower the temperature, the smaller was the slope. Therefore, the growth temperature after transfer and the slope [fresh weight versus the content of 16:1(3*t*)] displayed a perfect linear correlation y=0.99x−7.51 (r=0.99). The extrapolation of the straight line to the slope zero corresponded to a growth temperature of 7.5°C.

Effect of growth temperature on the 16:1(3t) content to chlorophyll ratio as a function of seedling growth—Chlorosis is a common sympton of chilling injury in several chilling-sensitive plant species, namely maize (Schapendouk et al. 1989). Therefore, chlorophyll content is frequently used to assess chilling damage (Schapendonk et al. 1989, Hugly et al. 1990). Under the conditions used in this study it was of interest to compare the relative increase of 16:1(3*t*) during seedling growth on the basis of chlorophyll. Fig. 8 shows that in control plants (30°C) the nmol 16:1(3*t*)/mg chlorophyll ratio increased slightly during the first two days of growth (from about 20 to 35) then remained constant. At lower temperatures (18°, 14° and 10°C), the ratio increased progressively to reach after 6 d of growth a value which was 3.5 times higher than the initial one.

Discussion

Growth restriction and leaf chlorosis are the common symptoms of chilling injury in chilling-sensitive plants (Lyons 1973, Schapendonk et al. 1989, Hugly et al. 1990). Concerning the lipid metabolism, the fatty acid composition of PG, compared to the other lipid classes of thylakoids, is the most affected by temperature, not only at injurious (Table 1; Fig. 2, 3) but also at non-injurious temperatures (Xu and Siegenthaler 1996b).

To investigate the role of PG in chilling-damage, we have studied the changes of fatty acid composition induced by low temperatures and compared them with the extent of growth and chlorophyll bleaching. Our results show that when plants were grown at 30°C (control plants), the fresh weight of cotyledons (Fig. 1) as well as the relative content of 16:1(3*t*) expressed in mol% (Fig. 3) or nmol per mg chlorophyll (Fig. 8) increased during the first two days of growth. All these parameters reached their maximal level after two days, whilst the relative 18:3 content (mol%) did not change during growth (Fig. 2). In contrast, when plants were transferred to lower temperatures the increase of cotyledon fresh weight was gradually diminished whereas the relative contents of 18:3 (mol%) and of 16:1(3*t*) [mol% and nmol per chlorophyll] increased. Interestingly, when plants were grown at 10°C, the growth of cotyledons almost stopped, although 18:3 and 16:1(3*t*) increased dramatically. This indicates that even at chilling temperature, the PG desaturases in thylakoids of squash cotyledons were still active. Altogether, these results suggest that in thylakoid membranes chlorophyll accumulation and growth (expressed by fresh weight increase) are more sensitive to low temperatures than the desaturation of PG leading to 18:3 and 16:1(3*t*) molecular species (Xu and Siegenthaler 1996a). Indeed, it was reported that chill-induced chlorosis in maize seedlings is partly the result of two metabolic blocks in the porphyrin pathway leading to chlorophyll synthesis and that the temperature range of the impaired chlorophyll synthesis coincides with that of chlorosis, i.e. from 17° to 10°C (Hodgins and Huystee 1986).

Changes in the lipid composition induced by low temperature have been observed in a variety of plant and cyanobacteria membranes. These changes are generally considered to be one of the main factors conferring low temperature tolerance by keeping the adequate membrane fluidity (Vigh et al. 1993, Murata and Wada 1995, Kodama et al. 1995). In accordance with this concept, we found that low temperatures induced always an increase in 18:3, mainly at the expense of 18:1 and to a lesser degree of 18:0 and 18:2 in the thylakoid PG of squash cotyledons. However, low temperatures induced also an increase in 16:1(3*t*) (Fig. 3). This fatty acid is known to display physical properties (e.g. high melting point, configuration, etc.) which are similar to those characterizing 16:0 (Bishop and Kenrick 1987). Moreover, the relative increase in the content of PG-16:1(3*t*) induced by low temperature was greater than that of PG-18:3. For example, when squash plants were transferred from 30° to 14°C for 6 d (Fig. 2, 3), the thylakoid PG of cotyledons contained 8.0 mol% more 16:1(3*t*) and 5.3 mol% more 18:3 than the corresponding thylakoid PG

from plants kept at 30°C for the same time. This difference suggests that 16:1(3*t*) may contribute to the formation of not only 18:3/16:1(3*t*) PG molecular species but also of other species such as 16:0/16:1(3*t*), as found recently in our laboratory (Xu and Siegenthaler, unpublished data). Thus, the conversion of 16:0 to 16:1(3*t*) induced by low temperature in thylakoid membranes of squash cotyledons does not result in an increase of membrane fluidity. At this stage, it is therefore risky to claim that low-temperature induced PG desaturation always leads to an increase of the membrane fluidity.

The above results could be explained by a mechanism involving the synthesis of the different molecular species of PG in the chloroplast. The fatty acid synthesis in plant cells takes place exclusively within the plastid (Ohlrogge and Browse 1995). The final products of the synthesis of fatty acids are 16:0 and 18:1. After these fatty acids have been incorporated into a PG molecule, 18:1 at the *sn*-1 position of the glycerol is desaturated into 18:2 and 18:3 (Ohlrogge and Browse 1995), whilst 16:0 at the *sn*-2 position is desaturated into 16:1(3*t*) (Ohnishi and Thompson 1991). Thus, 18:1- and 16:0-containing PG are the initial products of PG synthesis whilst 18:3- and 16:1(3*t*)-containing PG can be considered as the final desaturation products of PG. Both types of PG are constituents of thylakoid membranes. Our results show that squash plants grown at 30°C, contained low levels of 16:1(3*t*) and 18:3 and high levels of 16:0 and 18:1 in their thylakoid PG. When plants were transferred from 30°C to lower temperatures (18°, 14° and 10°C), both 18:3 and 16:1(3*t*) contents increased indicating that low temperatures induce an increase in the final products of PG at the expense of the initial ones. This increase is unlikely to be due to a higher absolute activity of the desaturases because the formation rate of 18:3 and 16:1(3*t*) was gradually limited by lowering the temperature within the 10° to 18°C range (Fig. 2, 3). Alternatively, these changes might reflect that the rates of fatty acid synthesis and PG desaturation are differentially affected by low temperatures, i.e. the fatty acid synthesis appears to be more strongly impaired by low temperature than the desaturation reactions.

Another interesting feature of this investigation is the finding that each squash plant grown at a given temperature was characterized by its own ratio of cotyledon fresh weight and relative content of 16:1(3*t*) in thylakoid membranes (Fig. 6). For instance, slopes of the straight line characterizing this relationship (or ratio) was increased as a function of growth temperature. Thus, a low temperature affects more significantly the fresh weight (which reflects the global physiological activity of the plant) than the conversion of 16:0 to 16:1(3*t*) (which is the expression of a membrane-bound lipid desaturase). Interestingly, the extrapolation of the straight line to zero (slope=0) displayed a temperature equal to 7.5°C (Fig. 7). At this temperature, the growth of squash cotyledons was completely abolished. In conclusion, the sensitivity of squash cotyledons towards temperature can be characterized by the equation of the above straight line (i.e. growth temperature versus slope): $y=0.99x+7.51$. This equation takes into consideration two parameters: the global physiological activity of the plant (i.e. the fresh weight) and a specific membrane bound enzyme (i.e. a lipid desaturase). One can expect that each plant is characterized by a different equation. We are currently investigating this hypothesis.

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